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Research Article

EFFECT OF LEAD NITRATE, ADMINISTERED ON THE HISTOLOGY OF KIDNEY OF *CYPRINUS CARPIO* (COMMON CARP)

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ABSTRACT

Acute toxicity (96 h LC50) of lead nitratel was analyzed in the *Cyprinus carpio* in static bio-assay over a 96-h exposure period using probit method. The aim of this study was to assess the effect of lead nitrate, administered on the histology of kidney of *Cyprinus carpio*. Fish were exposed to sub-lethal concentration of lead nitrate (5 ppm) over a period of 15, 30, 60 and 90 days. In this study, various groups with one control and experimental each of 10 fingerlings of common carp were used. Investigations pertaining to the histopathological sections have shown high degree of pathological lesions observed with the passage of time. Study showed dermal necrosis, disintegration of parietal layer of glomerulus, disorganization of proximal tubule with pycnotic nuclei and excessive increase in hematopoietic tissue that spread over large area. The nuclei of renal tubules were hyperchromatic and in several cells were displaced to an apical position, Exposure of fish to lead nitrate for showed increased disintegration of glomerulus, increase in Bowman's space of glomeruli, vacuolated proximal tubules, enlarged basement membranes of proximal and distal tubule and increased hematopoietic tissue. The results of the present study clearly indicate that chronic exposure to lead nitrate caused adverse effects on the kidney of *Cyprinus carpio* and also the quality of water.

Keywords: *Cyprinus carpio*, Pb(NO3)2, LC50 values, Liver, Kidney, Histopathology, Toxicity, Hypercromatic.

INTRODUCTION

Lead's industrial significance stems from its unique combination of physical and chemical properties. Due to the impending energy crisis, there is a need for battery production in which lead is an essential constituent, which was previously used to prepare paints and pigments. However, due to the phenomenon of bioaccumulation, many nations have now restricted its use (WHO, 1995). The surface water has a concentration of 0.02 g/L, which varies by a few micrograms/L. Indeed, elevated lead loads are associated with regions near lead mines, smelteries, and battery-producing factories. Toxic metals released from household, industrial, and other human operations may contaminate water systems broadly. Toxic metal pollution may have a negative impact on the ecological equilibrium and biodiversity of marine environments (Vinodhini & Narayanan, 2007). Lead is a major aquatic pollutant in many parts of the globe, making it one of the heavy metals, fluctuates by a few micrograms/L, Indeed, elevated loads of lead are allied with areas in the locality of lead mines, smelteries and battery-producing factories. Water systems may be widely contaminated by toxic metals released from household, industrial, and other human activities. Toxic metal pollution may have detrimental effects on ecological balance and biodiversity of marine ecosystems (Vinodhini & Narayanan, 2008). Among the heavy metals, lead is a major aquatic pollutant in many parts of the world. The natural waters are persistently being polluted by lead due to increased anthropogenic activities and industrial utilization. Lead is a ubiquitous metal in the environment and is toxic to aquatic organisms. Major sources of this toxicant include mining, smelting, coal burning, cement manufacturing, storage batteries, paints and use in gasoline The unregulated release of agricultural chemicals into water bodies has caused environmental problems to all classes of organisms in the aquatic habitat (Deng *et al*., 2017; Chai *et al*., 2017). The aquatic ecosystem has a great importance in the Indian economy. Fishes serve as a bio-indicator of

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aquatic ecosystem and fishes accumulate both directly and indirectly the pollutant from water and food chain. Hence, the disposal of pollutants in water poses adverse effect on the fish health and other aquatic organism, As a consequence, fish production is generally hampered, and fishermen suffer significant economic losses. As a result, it is critical to watch and comprehend pollutant pathophysiology. To mitigate the negative consequences and protect public health, The toxic effects of different heavy metals on fish growth rates, physiological functions, mortality, and reproduction are all possible (Ebrahimi and Taherianfard, 2011).Worldwide great attention is given to the studies of toxicity of heavy metals in fish with the increase of pollution in aquatic bodies and pisciculture. The toxicity of lead nitrate in fish has been reported by several authors (Khidr and Mekkawy, 2008; Devi and Banerjee, 2007; Osman *et al*., 2008). However, there is a lack of study in the field of genotoxicological manifestation and assessment of water quality in lead nitrate-exposed fish. The Common carp, *Cyprinus carpio* (L., 1758) is a freshwater cyprinid fish that is widely farmed fish distributed in the world. It occurs in shallow ponds, lakes rich in vegetation and slow moving rivers. It burrows in mud in the dry season or winter and tolerates cold, organic pollutants and low oxygen concentration in water (Weber et al, 2010). The aquaculture system has developed rapidly during the last decades playing an important role in contributing to the protein demands for humans via the amplification of fish farming. Common carp, *Cyprinus carpio* L., is one of the most cultivated carp species throughout the world because of its high growth, breeding in confined waters, hardy nature, and easy adaptation to artificial feeding (Khan *et al*., 2016; Abdel-Tawwab and Monier 2018; Iswarya *et al*., 2018; Monier, 2020).

The present work investigates the toxic effect of lead nitrate on freshwater fish *Cyprinus carpio*. On the other hand, water quality is an important factor, which directly affects the growth rate, fish's health, survival and economic as well as public health. These heavy metals ultimately reaches the water bodies and adversely affect the growth, reproduction, physiology and survival of aquatic life, the non-target organisms, including major carps due to their stable and persistent existence in the environment (Hayat *et al*., 2007). As in higher vertebrates, the kidneys of fish perform an important function related to electrolyte, water balance and maintenance of a stable internal environment. The kidney of fish receives the largest proportion of post branchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution. The histopathology is often the easiest method for assessing both the short term and long term effects of the toxicant and thus acts as biomarker for complete evaluation of the organism's health. However, histopathology in toxicity studies does require the ability to discriminate between toxicant induced lesions and normal variation in structure. If the normal appearance of various organs and tissues of fish is known, alterations from the expected may indicate stressor related injury (Hinton and Lauren, 1990). Several histological alterations have been reported in the muscle, gills, liver and kidney of fish in response to industrial, sewage and agricultural pollutants. Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. Previous studies reported that the exposure of fish to pollutants (agricultural, industrial and sewage) resulted in several pathological alterations in different tissues of fish (Abbas and Ali, 2007). Kidneys are pivotal organs of the body responsible to maintain the homeostasis as liver is center of metabolism and detoxification, while kidneys are involved in elimination of the wasteful chemicals from body and selective reabsorption (Iqbal *et al*., 2005; Palacios and Risbourg, 2006). Accumulations of the chemical pollutants are known to adversely affect the histology and functioning of liver, kidneys, muscles and other organs of fish (Iqbal *et al*., 2004). Histopathology is considered an ultimate tool to find out the effect of pollutants like copper and lead on fish tissue, because the heavy metals are active toxicants for the normal physiology of the animal (Atamanalp et al., 2008; Pathan et al., 2009). One study has suggested that lead can enter into fish by infiltration through gills, skin and the digestive tract (Farombi *et al*., 2007; Łuszczek-Trojnar *et al*., 2013; Bashir *et al*., 2020). Lead accumulates in fish and is a major cause of serious health issues in human consumers (Ibrahim *et al*., 2018; Javed and Usmani, 2019). Fish act as biological indicator of aquatic pollution. Analysis of biochemical components of fishes very helpful for the study of water quality and therefore ensures safety food for people. Now days the assessment of environmental pollution cannot be based exclusively on chemical analysis since the toxic effect of pollutant does not provide a clear indication on the aquatic organisms. The unequivocal conclusion of ecosystem health requires the higher aquatic organisms. The present study was conducted to sort out the after effects of the dissolved elements in water bodies. Common carp being being frequently used as food by public.

MATERIALS AND METHODS

Fish and acclimatization conditions

Cyprinus carpio (Linnaeus 1758) is an exotic fish to India, generally known as scale carp. It is an extensively cultivated species and is commercially important. *C. carpio* is voraciously omnivorous and grows fast on artificial fish feed. *Cyprinus carpio* of weight and length measuring 80-120gm and 18-20 cm respectively were obtained from the local fish market and brought to the laboratory. The fish were kept in the glass aquarium to observe any visible pathological symptoms. Before introducing in the aquarium fish were treated with0.1% $KMnO4$ solution to obviate any dermal infection. Fish were acclimatized to laboratory conditions for a period of 15 days. Both control and treated fish were fed pallets daily. The fish were divided into groups kept in three transparent glass aquariums (120l), group I served as the control and others were treated with lead nitrate concentrations of 5, 10, 15, 20 mg/l respectively. In order to maintain the constant

concentration of lead nitrate water was changed after 48 hours during the experimental period.

Using Probitanalysis and SPSS

Statistical analysis software, the actual LC50 data was examined. Probit analysis is based on linear regression technique after transformation of toxicity data in the parametric procedure and extensive history of statistical applications.

Calculation of LC 50 value

Seven different concentrations of Lead nitrate were used to access its effect on the mortality of *Common carp* and to determine the LC50 of the Lead nitrate, during the present experimental period. 10 mg/l to 60 mg/l Lead nitrate was used to assess the rate of mortality at each concentration. 60 mg/l was seen to be LC100 for Lead nitrate. 50% mortality was recorded in experimental groups exposed to 30-40 mg/l of Lead nitrate (Graph1). Hence the acute 96h LC50 value for the present experimental fish, *Common carp* was calculated to be 35 mg/l (ppm). The numeric calculation of LC50 of Lead nitrate for *Common carp* is presented in table 1.

Histological Procedure

Both treated and untreated fish were stunned by a blow on the head at the end of the specific experimental time duration. The fishes were dissected and slices from the kidney were immersed in fixative Bouin's fluid Preserved tissues were washed under tap water and dehydrated; clarified with xylene and embedded in paraffin blocks. They were cut at 4-5 µ thickness by using microtome and stained routinely with haematoxylin and eosin (H & E) for histological examination (Luna, 1968). Stained histopathological sections were examined under binocular microscope on different magnifications and photographed

RESULTS AND DISCUSSION

The kidney of *Cyprinus carpio* comprises of functional units, the nephrones. Each of which consists of a renal corpuscle and a renal tubule. The renal corpuscle of nephrone consists of glomerulus and Bowman's capsule. A tubular neck follows the Bowman's capsule. Other regions of the renal tubule are proximal distal and collecting tubules. The interstices of the tubules are enriched with hematopoietic tissue, which contain round to polygonal cells possessing hyperchromatic nuclei. The control fish (untreated) (Figure 1) shows normal appearing renal parenchyma with intact tubules and glomeruli. The heavy metal accumulation in fish depends on both the structure and exposure of the tissues and organs to the environment. Exposure of *Cyprinus carpio* to 5 ppm lead nitrate for a period of 15 days showed no major morphological change in the kidney tissue (Figure 2) shows the kidney renal parenchyma with tubular necrosis and glomerular shrinkage. In vacuolated cytoplasm, shrinkage in glomerulus, and enlarged Bowman's capsule after 15 days of exposure to lead nitrate, however, progressive deteriorating changes in kidney histology were noticed. 30 day exposure (Figure 3) of fish to lead nitrate showed disintegration of parietal layer of glomerulus, disorganization of proximal tubule with pycnotic nuclei and excessive increase in hematopoietic tissue that spread over large area. The nuclei of renal tubules were hyperchromatic and in several cells were displaced to an apical position. Exposure of fish to lead nitrate for 60-90 days showed increased disintegration of glomerulus, increase in Bowman's space of glomeruli, vacuolated proximal tubules, enlarged basement membranes of proximal and distal tubule and increased hematopoietic tissue (Figure 4-5). The collecting duct or glomerulus was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border (Peebua *et al*., 2006). Results of this study demonstrated that concentrations of lead nitrate produce a variety of histopathological changes in the kidneys of exposed fish.

The present study corroborates the findings of (Sharma and Tamot, 2015). The common alterations found in the kidney of treated fish were severe degenerative and necrotic changes in the renal tubules and edema in Bowman's capsules with atrophy in the glomeruli. The present results are in agreement with those observed in fish exposed to lead acetate (Al-Balawi *et al*., 2013). Environmental pollution with heavy metals can cause serious effects such as genetic abnormalities, physiological and biochemical problems and behavioural disorders in aquatic organisms (Scott *et al*., 2003). Fish are unique amongst the vertebrates, because there is the potential for nutritive metal acquisition from the water in addition to metal absorption from the diet (Nicolas and Martin, 2003). Various metals are accumulated in fish body in different amounts due to different affinity of metals to fish tissues, different uptake, deposition and excretion rates (Jezierska and Witeska, 2009). The sequence of behavioral responses of *Cyprinus carpio*, such as abrupt swimming and sluggish movements on exposure to nitrate agree well with the known effects of various toxicants on different animals (Brungs *et al*. 1973, Kumari and Benerjee 1986). In Bowman's space, degeneration of glomeruli, shrinkage of proximal tubule cells with pycnotic nuclei, increased tubular lumen and increased in intratubular hematopoietic tissue were the most significant changes observed in fish kidney after nitrate exposure. The effects were time dependent being more pronounced in acute treatments. The present investigation illustrates that presence of high concentration of nitrate in water are stressful to fish (Iqbal *et al* 2004;Camargo and Martinez, 2007) studied that the sub-lethal effects of endosulfan on histological alterations found in the brain, gills, intestine, and liver of *C. carpio.* Fish gills have a direct connection with the external environment; that is why they are the primary target of contaminants. Gills also perform various relevant and useful functions like acid-base balance, respiration, osmoregulation, and excretion; therefore, in ecotoxicological research gills, morphology can be used as

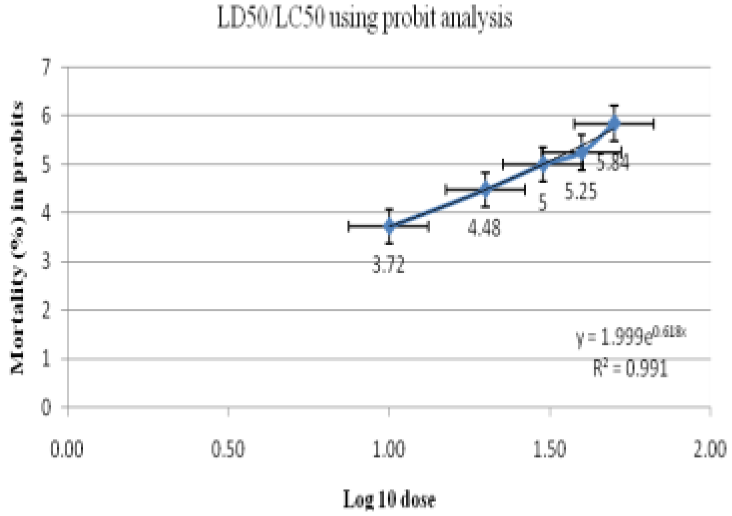
a biomarker. Number of pollutants enter the aquatic ecosyste with domestic, industrial and agricultural wastewaters and severely affect the aquatic organisms. With the increase in industrialization, freshwater resources are getting more polluted day by day (Chavan and Muley, 2014). The water quality affected the production traits of fish in aquacultures (Davies and Ansa, 2010).

In general, fish appear to acclimatise in chronic settings with sublethal nitrate exposure. Cultivating fish in water with excessive nitrate levels can have serious consequences for the fish's overall health and culture. While fish was employed as a model physiological system in these tests, similar results could be expected in human populations as a result of nitrate exposure, which could lead to glomerulonephritis and other disorders that need to be studied more in the future. Pollutants from home, industrial, and agricultural wastewaters penetrate the aquatic environment and have a negative impact on aquatic life. Freshwater resources are becoming increasingly polluted as industrialization progresses (Chavan and Muley, 2014), which is a major source of water pollution. The uncontrolled dumping of agricultural chemicals into bodies of water has generated environmental issues for all kinds of creatures in the aquatic ecosystem (Deng *et al*., 2017; Chai *et al*., 2017). The kidneys of *Labeo rohita* subjected to lead nitrate showed significant congestion in blood vessels and tubular necrosis of the glomerulus. Significant histological changes were observed in the gills, liver, brain, and intestinal tissue of common carp subjected to varying concentrations of endosulfan. Endosulfan has a considerable influence on haematological, biochemical, and histopathological parameters of common carp during acute poisoning, according to the findings (Khan *et al*., 2019).The current study is thus a pioneer work on the histopathological changes in the kidney of *Cyprinus carpio* because no record of histopathological changes in any organ of a fish due to nitrate exposure was accessible.

The primary organ for lead buildup in fish is the kidney. In this study, histological changes in the kidney included glomerulus shrinkage, damaged haemopoietic tissue, and nuclei of uriniferoustubules migrating towards the tubular lumen. Cytoplasmic vacuolation in tubular cells, glomerulolysis, enlarged Bowman's gap, and necrosis are all symptoms. The accumulation of heavy metals in fish organs is the major cause of structural damage, toxic effects on physiology, and metabolic defects (Thophon *et al*., 2003). Similarly, Das and Mukherjee (2000) found extravasation of blood from blood vessels, coagulative necrosis of the first and second proximal segments, irregular blood congestion, and tubular necrosis in fish kidneys treated with a mixture of copper sulphate and lead nitrate. The authors mentioned previously stated that contaminants that are transported from the blood to the tissues cause significant harm to the cellular architecture and may disrupt normal tissue function. Necrosis, which often denotes cell death in response to toxicant exposure, causes space to be emptied, followed by vacuolation (Javed *et al*., 2017).

Figure 1. Culture Tank in *Cyprinus carpio.*

Figure 2. Illustration of experiment setup.



Graph 1. LD50/LC50 in *Cyprinus carpio* exposed to various concentrations of Lead Nitrate

Table 1. The Lead Nitrate concentrations, groups and calculations of the acute 96 h LC50 value of Lead Nitrate in *Cyprinus carpio.*

Lead Nitrate Conc. (mg/l)	No. of Fish	No. of Dead Fish	A	B	axb
0.00	10	0	0	0	0
10.00	10	1	10	0.5	5
20.00	10	3	10	1.5	15
30.00	10	5	10	3	30
40.00	10	6	10	5	50
50.00	10	8	10	6.5	65
60.00	10	10	10	8.5	85
			∑axb = 250		
	70		LC50 = 35 mg/l		

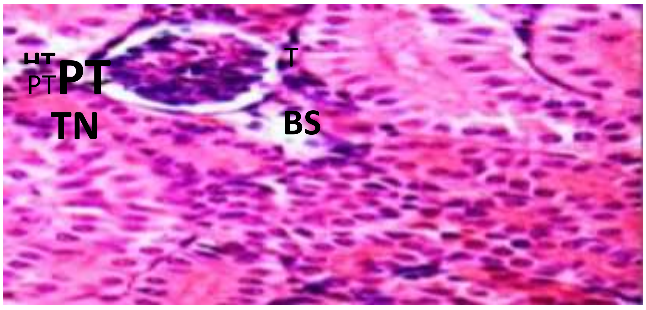


Figure 1. Photomicrograph depicting collecting tubule, distal tubule, glomerulus, hematopoietic tissue, proximal tubule of kidney of untreated fish. H & E. x 1280.

Control No recognizable changes were observed in the kidneys of the control fish (Figure 1). The kidney was composed of numerous renal corpuscles with well-developed glomeruli and a system of tubules surrounded by hematopoietic tissue.

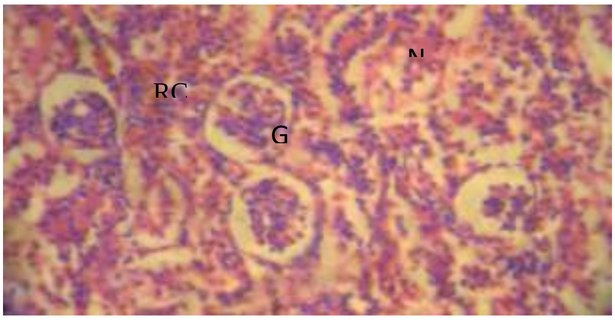


Figure 2.Photomicrograph of kindney of common carp exposed to 5mg/l of lead nitrate for the period of 15days disintegration of parietal layer of glomerulus (DPLD), excessive increase in hematopoietic tissue (HT).

Histological alterations in the kidney of common carp exposed to 5mg/L lead nitrate for the period of 15 days. Exposure of *Cyprinus carpio* to 5 ppm lead nitrate for the period of 15 days showed some morphological change in the kidney tissue, like increased Bowman's space and necrotic damages were seen in hematopoietic tissue and glomerulopathy and dilations of Bowman's capsule were also prominently seen in figure 2

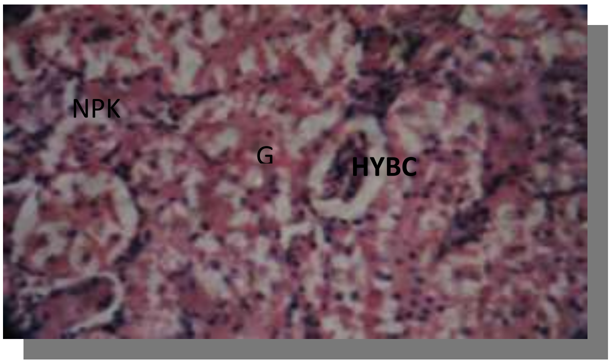


Figure 3. Photomicrograph of kidney section of common carp showing disintegrated glomeruli, disorganized vacuolated and pycnotic proximal and excessive increased hematopoietic tissue(HT) in fish exposed to 5ppm lead nitrate for 30 days.

Histological alterations in the kidney of common carp exposed to 5mg/L lead nitrate for 30 days. On the 30th day of exposure the kidney of common carp showed progressive deterioration and disintegration in the parietal layer of glomerulus, disorganization in the proximal tubule, pycnotic nuclei and excessive increase in hematopoietic tissue , and nuclear piknosis of renal tubules were seen visualized.

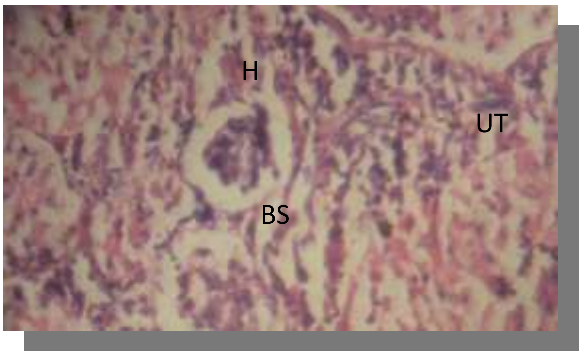


Figure 4. Photomicrograph of kidney section of common carp showing disintegrated glomeruli, disorganized vacuolated(V), pycnotic proximal and excessive increased hematopoietic tissue(HT) in fish exposed to 5ppm lead nitrate for 60 days

Histological alterations in the kidney of common carp exposed to 5mg/L lead nitrate for 60 days. On the 60th day of exposure of lead nitrate increased disintegration of glomerulus, increase in Bowman's space , vacuolated proximal tubules, enlarged basement membranes of proximal and distal tubule and show increase in hematopoietic tissue.

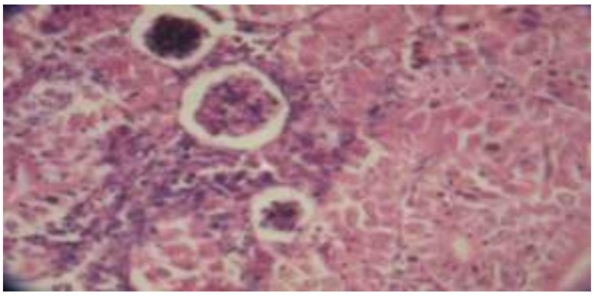


Figure 5. Photomicrograph of kidney section of common carp showing disintegrated glomeruli, disorganized vacuolated(V), pycnotic proximal and excessive increased hematopoietic tissue(HT) in, and vacuolated (PT) fish exposed to 5ppm lead nitrate for 90 days

Histological alterations in the kidney of common carp exposed to 5mg/L lead nitrate for 90 days. Similar as that of in 60th day of exposure the kidney sections of common carp showed increased disintegration of glomerulus, Detached epithelium from lamina bowman's space, nuclear piknosis of renal tubules, increase in Bowman's space, vacuolated proximal tubules, enlarged basement membranes of proximal and distal tubule and increase in hematopoietic tissue (HT).

CONCLUSION

These findings of our study demonstrations show that chronic $Pb(NO_3)2$ exposure caused a destructive impact in the kidney tissues, which may eventually lead to fish death, and having indirect impact on human health because this fishes are an important component of the human food chain. These Histopathological studies revealed that modifications have been observed in the structural patterns of kidney cells indicate that *Cyprinus carpio*(common carp) is a potential experimental indicator for the aquatic pollution and could be a suitable species for examining water quality parameters kidney functions are useful for the deduction of nephrotoxicity induced by these heavy metals.

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